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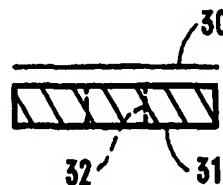
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(54) Title: **BLOOD TESTING KIT**

(57) Abstract

An improved blood testing kit and method of testing blood is provided. The improved kit may be provided in two or more forms including a strip from whereby a membrane (30) and an absorbent pad (31) are attached to a backing sheet in a juxtaposed relationship with one another. A portion of one side of the membrane (30) is treated with antibodies that will react with the antigens for A type, B type and RH+ blood. Another embodiment includes the placement of a membrane over an absorbent pad (31) within a housing. A hole or aperture (32) is disposed in the pad (31) underneath a portion of the membrane (30). Discrete portions of the membrane are treated with antibodies specific to the A type, B type and RH+ blood antigens. If the antibodies are disposed on the underside of the membrane, a membrane having a pore size of approximately 20 microns is utilized. If the antibodies are disposed on the top or upper surface of the membrane (30), a membrane having a smaller pore size of 3-8 microns is utilized.



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## BLOOD TESTING KIT

## FIELD OF THE INVENTION

5        This invention relates generally to apparatus for testing blood including tests for blood type or viral infections, such as HIV. More specifically, this invention relates to the use of membranes in blood testing apparatuses.

## BACKGROUND OF THE INVENTION

10       Blood testing is normally performed in a laboratory setting requiring several steps for each type of test to be performed. While the present invention is directed toward two basic type of blood tests, blood type testing and testing for viral infections,  
15       other applications will be apparent to those skilled in the art. Blood typing is performed by detecting the type of antigen that is contained within a person's blood. Specifically, a person with A type blood has the A antigen in their blood cells; people with B type blood have the B antigen in their blood cells; people with AB  
20       type blood have both the A antigen and the B antigen in their blood cells; and people with O type blood have neither the A nor the B antigen.

      Most prior art methods of typing blood rely upon an agglutination process whereby the red cells agglutinate in the  
25       presence of a specific antigen type antibody. Specific antigen type antibodies exist for all blood types except type O. For example, A type red blood cells will react with a specific antigen type antibody which results in a clumping or aggregation of the A type blood cells and the A type antigen antibodies. Thus, if A  
30       type blood is exposed to the specific antigen antibody for A type blood, the result is a clumping of A type red blood cells and the antibody. The visual appearance of the clumping is a positive indication that the blood is A type. Similarly, clumping will occur when B type red blood cells are exposed to B type antibodies.  
35       In the case of AB type blood, the blood sample must be exposed to

both A type antibodies as well as B type antibodies. The clumping reaction for both types of antibodies is a positive indication for AB type blood. Finally, in the case of O type blood no clumping will occur when exposed to A type or B type antibodies. Similar  
5 agglutination methods are used for RH factor determination. A schematic diagram illustrating the agglutination method is presented in Figure 1.

Another process utilizes size exclusion chromatography. In this method, a mixture of blood and antibody is placed in a test  
10 tube on top of a layer of gel. The tube is then placed in a centrifuge. If the sample contains red cells with the specific antigen on their surface, the cells and antibody will clump together and will not be able to be centrifuged through the gel. If the sample does not contain red cells with the specific antigen  
15 on their surface, the cells will easily be centrifuged through the gel. Therefore, red blood cells that are centrifuged through the gel is a negative reaction and a band of red cells disposed on the top of the gel after centrifuging is a positive reaction. The size exclusion chromatography method is illustrated in Figure 2.

20 Until now, chromatography was the only method of size exclusion blood typing or blood testing. Membranes, if used at all, were merely used for an indication of a positive reaction at the surface of the membrane. However, with recent advances in membrane technology, it would be particularly useful to utilize  
25 membranes in a size exclusion blood testing method.

Specifically, the antigen or reactant could be disposed on an underside or an opposing side of a membrane. Blood then could be forced to proceed through the membrane before reacting with the antigen or other reactant. The remaining components of the blood  
30 could be thereafter absorbed or washed away leaving the blood cell-reactant aggregate disposed against the underside of the membrane. Because membranes are clear, the result could be an easy-to-read indicator such as a line or a dot. Further, separate antigens or reactants could be placed at discrete locations on the  
35 membrane to provide multiple indication in one test kit. For example, both A type and B type as well as RH positive antibodies could be placed on discrete areas of the membrane so that one test apparatus could provide positive indications for A, B, AB, O, RH

positive and RH negative blood. Such a system would not require the use of a centrifuge or excessive handling by a technician.

#### SUMMARY OF THE INVENTION

5 The invention is an improved apparatus for testing blood, such as testing blood type and testing for viral infections. One apparatus made in accordance with the present invention includes a backing sheet for accommodating a membrane on one surface section of the backing sheet and an absorbent pad on a second surface section of the backing sheet. The membrane and absorbent pad are in a juxtaposed relationship with one another. On the underside of the membrane, antibodies corresponding to A, B and RH positive blood cells are arranged in discrete areas of the membrane. One preferred method is to place a horizontal "stripe" of antibody corresponding to each blood type in a parallel relationship. The backing sheet, absorbent pad and membrane are then cut into strips. The purpose of the absorbent pad is to absorb liquid as it flows through the membrane. When the blood cells react with an antibody or other reagent, the aggregation is too large to pass through the membrane and a red stripe appears where the antibody or reagent was placed on the membrane.

A second and related embodiment features a membrane mounted onto the top of an absorbent pad. The absorbent pad includes a hole or aperture disposed in the middle of the pad. The membrane/pad combination are then accommodated in a housing which includes an opening or access hole disposed above the portion of the membrane covering the hole or opening in the absorbent pad. The appropriate antibodies or reagents are previously placed in discrete areas of the membrane portion which is disposed over the hole in the pad. Blood is then applied directly to the top of the membrane and subsequently washed away with a buffer solution. Blood cells which react with the antibodies or reagents form an aggregation or bright red area after the remaining components of the blood are washed away with the buffer solution.

35 A membrane having approximately a 20 micron pore size is preferable for use with both blood testing apparatuses described above. Red blood cells are approximately seven to eight microns in diameter. Thus, unreacted red blood cells will pass through the 20 micron membrane. However, once the red blood cells react with

an antibody or other suitable reagent to produce an aggregate, the aggregate cannot pass through the 20 micron pore size. As the remaining components of the blood are washed away with a buffer solution, the aggregate combination is left behind to provide a bright red indicator on the under surface of the membrane. Because the membranes are transparent, a clear visual indication is provided.

In contrast, it may be desirable to allow the blood and reagents to aggregate on the surface of the membrane. In such a situation, a membrane with a smaller pore size, such as 3-6 microns is provided so that the blood and reagents will bind to the surface of the membrane.

It is therefore an object of the present invention to provide an improved apparatus for testing blood.

Yet another object of the present invention is to provide an improved blood-type testing kit.

Still another object of the present invention is to provide an improved apparatus for testing viral infections in human blood.

Another object of the present invention is to provide an improved method for testing blood.

Other objects and advantages of the present invention will become apparent upon reading the following detailed description of the drawings and appended claims, and upon reference to the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated more or less diagrammatically in the accompanying drawing wherein:

Figure 1 is a schematic diagram illustrating a positive reaction between an antibody and a red blood cell with the specific antigen and a negative reaction between an antibody and a red cell without the specific antigen;

Figure 2 is an illustration of the prior art method of using size exclusion chromatography in testing blood;

Figure 3 is a top plan view of a plastic backing sheet used in one blood testing apparatus made in accordance with the present invention;

Figure 4 is a top plan view of the plastic backing sheet shown in Figure 3 with a membrane applied to a lower surface section thereof;

Figure 5 is an illustration of the backing sheet and membrane shown in Figure 4 further with an absorbent pad attached to the backing sheet in a juxtaposed relationship with respect to the membrane;

Figure 6 is an illustration of the embodiment shown in Figure 5, cut into strips and illustrating the reactions for A-, A+, B-, B+, AB-, AB+, O- and O+ blood types;

Figure 7 is a side view illustrating the attachment of a membrane to an absorbent pad for a second embodiment made in accordance with the present invention;

Figure 8 is a top plan view of the absorbent pad first shown in Figure 7;

Figure 9 is a side sectional view illustrating the membrane and absorbent pad shown in Figure 7 contained within a housing; and

Figure 10 is an illustration of eight blood testing apparatus shown in Figure 9 indicating A-, A+, B-, B+ AB-, AB+, O- and O+ blood types.

It should be understood that the drawings are not necessarily to scale and that the embodiments are sometimes illustrated by graphic symbols, phantom lines, diagrammatic representations and fragmentary views. In certain instances, details which are not necessary for an understanding of the present invention or which render other details difficult to perceive may have been omitted. It should be understood, of course, that the invention is not necessarily limited to the particular embodiments illustrated herein.

#### DETAILED DESCRIPTION OF THE DRAWINGS

Like reference numerals will be used to refer to like or similar parts from Figure to Figure in the following description of the drawings.

Turning to Figure 1, an antibody 11, a red blood cell 12 and a red blood cell 13 are shown at the left. The red blood cell 12 includes an antigen which will react positively with the antibody 11 which will result in an aggregation, shown generally at 14. The aggregation 14 is a positive reaction or a positive indication that

the red blood cell 12 is of the blood type specific to the antibody 11. In contrast, the antibody 11 will not react with the red blood cell 13 because the red blood cell 13 lacks the specific antigen. The combination of the antibody 11 and the red blood cell 13 is a negative reaction or lack of clumping, indicated generally at 15. The aggregation or agglutination shown at 14 is useful in size exclusion chromatography as shown in Figure 2.

A size exclusion chromatography method is illustrated in Figure 2. A test tube 16 is filled with a gel 17. A mix type of red blood cells and a specific antibody are placed on top of the gel 17 at the area indicated generally at 18. The test tube 16 is then placed in a centrifuge (not shown). If no reaction takes place between the antibody and the red blood cells, the red blood cells shown at 20 will centrifuge downward toward the lower end of the test tube below the gel, indicated generally at 19. The test tube shown in the middle of Figure 2 indicates a negative reaction, because the red blood cells 20 were able to pass through the gel 17 during the centrifuge process. In contrast, the red blood cells shown in the test tube 16 at the far right of Figure 2 reacted with the antibody and formed an aggregation 21 which was not able to pass through the gel 17 during the centrifuge process. As a result, the red blood cells in combination with the antibodies are shown at 21, disposed on top of the gel 17. The aggregation was unable to pass through the gel and therefore the area disposed at the bottom 19 of the test tube 16 remains clear.

The size exclusion chromatography process shown in Figure 2 is inconvenient for several reasons. Specifically, only one antibody or reagent may be used at a time. Thus, several tests are required to determine blood type which requires the use of several test tubes. Further, a centrifuge is required.

One embodiment of the present invention is illustrated in Figures 3 through 6. Figure 3 is an illustration of a backing sheet 22 which, as shown in Figure 4, accommodates a membrane 23. The dotted lines 24, 25 which pass through the membrane 23 are intended to indicate the placement of antibodies on either the top side or underside of the membrane 23. Figure 5 illustrates the placement of an absorbent pad 26 on top of the backing sheet 22 and in a juxtaposed relationship with respect to the membrane 23. As fluid passes through the membrane 23, it is absorbed into the pad



26. The apparatus 27 shown in Figure 5 can then be cut into strips 28a through 28h as shown in Figure 6.

Turning now to Figure 6, the membrane 23 has been coated with three specific antibodies -- the specific antibody for A type blood, the specific antibody for B type blood and the specific antibody for RH+ blood. As illustrated to the left of the strip 28a, the A type, B type and RH+ antibodies are coated on the membrane in spaced apart parallel stripes. The use of the three different antibodies enables the test strips 28 to indicate eight different results. Specifically, the test strip 28a gives a positive indication for A- blood. The test strip 28b is a positive indication for A+ blood. The test strip 28c gives a positive indication for B- blood; the test strip 28d gives a positive indication for B+ blood. Similarly, the test strips 28e and 28f gives positive results for AB- and AB+ blood respectively. Finally, the test strips 28g, 28h give positive results for O- and O+ blood respectively.

The test strips 28a through 28h as shown in Figure 6 can be used as follows: the blood samples are collected in a standard method with any type of anticoagulant to stop clotting. Approximately five microliters of blood is then added to a 12mm by 75mm test tube along with approximately 95 microliters of buffer containing 1% BSA, 50mM sodium phosphate ph 7.4, with 0.1 percent sodium azide. The strip 28 is then added to the tube with the membrane 23 on the bottom and the solution is allowed to migrate up the strip 28 to the absorbent pad 26. The results can then be observed within one to four minutes.

A second embodiment of the present invention is illustrated in Figure 7. Specifically, a membrane 30 is placed on top of an absorbent pad 31 as shown in Figure 7. As shown in Figures 7 and 8, the pad 31 includes a hole or aperture 32 over which the membrane 30 is placed. As shown in Figure 9, the membrane 30 and pad 31 are contained within an outer housing that includes a top 33 and bottom 34. Use of the apparatus 35 is further illustrated in Figure 10. Each membrane 30 includes three different antibodies, placed in discrete and separate positions, as illustrated by the kit 35f which indicates AB+ blood. The kit 35a indicates a positive reading for A- blood; the kit 35b indicates a positive reading for A+ blood. Similarly, the kits 35c, 35d

indicate positive readings for B- and B+ blood respectively. The kits 35e, 35f indicate positive readings for AB- and AB+ blood respectively. Finally, the kits 35g, 35h provide positive readings for O- and O+ blood respectively.

5       The kit 35 is used as follows. Fifty microliters of blood is placed on the top of the membrane 30. The blood is then allowed to sit for sixty seconds. The membrane is then washed with 1mL of the above-described buffer solution to remove the unreacted blood cells. The results as shown in Figure 10 are then interpreted.  
10       A red dot on the left hand side denotes A type antigen. A red dot on the right hand side denotes B type antigen. A red dot in the upper center portion of the membrane 30 indicates RH+.

      The preferred membrane is comprised of mixed esters of cellulose. The preferred pore size is approximately 20 microns.  
15       However, the pore size may vary. One preferred membrane is sold under the HIFLOW-SX™ which is sold in strips that are twelve inches wide, having a thickness of 115-155 microns. The top 33 and bottom 34 may be manufactured from plastic, such as PVC. The backing sheet 22 may also be manufactured from plastic.

20       Instead of utilizing a 20 micron membrane, a membrane having a smaller pore size, such as three microns may be utilized. If a 3 micron membrane is utilized, the antibodies should be applied to the top or front surface of the membrane. The reaction or aggregation will take place on the membrane's surface as opposed  
25       to the underside of the membrane as illustrated above. Finally, a 20 micron membrane may be utilized alone, without being fabricated into a strip form, such as the strips 28 as shown in Figure 6 or the kits 35 as shown in Figure 10. Specifically, ten microliters of blood may be mixed with twenty microliters of  
30       antibody solution with fifty microliters of buffer. This mixture may then be pipetted directly onto the membrane. A bright red spot will appear in positive samples as the agglutinated antibody red cell complex will not be able to pass through the twenty micron pore membrane. In a negative sample, the red cells pass through  
35       the membrane and no clumping is observed.

      The use of membrane in accordance with the present invention will be useful for other types of testing, other than blood-type testing. Specifically, the invention and methods of using the invention will be useful for the detection of viral infections,

such as HIV. Further, the present invention may be utilized to detect any type of a blood condition that may be otherwise detected by agglutination or aggregation.

Although only a limited number of embodiments of the present invention has been illustrated and described, it will at once be apparent to those skilled in the art that variations may be made within the spirit and scope of the invention. Accordingly, it is intended that the scope of the invention be limited solely by the scope of the hereafter appended claims and not by any specific wording in the foregoing description.

WHAT IS CLAIMED IS:

1. An apparatus for testing blood, the apparatus comprising:  
a backing sheet including a front surface thereof, the front  
surface of the backing sheet including a first section and a  
5 section;  
a membrane sheet being attached to the first section of the  
backing sheet, a first limited portion of the membrane sheet being  
coated with an antibody;  
an absorbent pad being attached to the section of the backing  
10 sheet, the membrane sheet and absorbent pad being in juxtaposed  
relationship with one another.
2. The apparatus of claim 1,  
wherein the membrane has a pore size ranging from about 3  
15 microns to about 25 microns.
3. The apparatus of claim 2,  
wherein the pore size about 20 microns.
- 20 4. The apparatus of claim 2,  
wherein the pore size about 3 microns.
5. The apparatus of claim 1,  
wherein the antibody is selected from the group consisting of  
25 antibody for A antigen, antibody for B antigen and antibody for RH  
positive antigen.
6. The apparatus of claim 5,  
wherein the membrane sheet includes a second limited portion  
30 that does not overlap said first limited portion, the second  
limited portion being coated with an antibody that is different  
than the antibody that the first limited portion is covered with.
7. The apparatus of claim 6,  
35 wherein the membrane sheet includes third limited portion that  
does not overlap said first and second limited portions, the second  
limited portion being coated with an antibody that is different  
than the antibodies that the first and second limited portions are  
covered with.

8. An apparatus for testing blood, the apparatus comprising:  
a backing sheet including a front surface thereof, the front  
surface of the backing sheet including a first section and a  
section;

5 a membrane sheet being attached to the first section of the  
backing sheet, the membrane including a pore size of approximately  
20 microns, a first limited portion of the membrane sheet being  
coated with an antibody selected from the group consisting of  
antibody for A antigen, antibody for B antigen, and antibody for  
10 RH positive antigen, a second limited portion that does not overlap  
said first limited portion, the second limited portion being coated  
with an antibody that is different than the antibody that the first  
limited portion is covered with, a third limited portion that does  
15 not overlap said first and second limited portions, the second  
limited portion being coated with an antibody that is different  
than the antibodies that the first and second limited portions are  
covered with;

an absorbent pad being attached to the section of the backing  
sheet, the membrane sheet and absorbent pad being in juxtaposed  
20 relationship with one another.

9. An apparatus for testing blood, the apparatus comprising:  
a bottom receptacle including an upper opening defined by an  
upper rim for accommodating a membrane sheet and an absorbent pad;  
25 the membrane sheet overlying the absorbent pad, a first  
limited portion of the membrane sheet being coated with an antibody  
selected from the group consisting of antibody for A antigen,  
antibody for B antigen and antibody for RH positive antigen;

the absorbent pad including an opening, the first limited  
30 portion of the membrane sheet being suspended over an area defined  
by the opening of the absorbent pad.

10. The apparatus of claim 9,  
wherein the membrane has a pore size ranging from about 3  
35 microns to about 25 microns.

11. The apparatus of claim 10,  
wherein the pore size about 20 microns.

12. The apparatus of claim 10,  
wherein the pore size about 3 microns.

13. The apparatus of claim 9,  
5 wherein the membrane sheet includes a second limited portion  
disposed within the opening of the absorbent pad, the second  
limited portion does not overlap said first limited portion, the  
second limited portion being coated with an antibody that is  
different than the antibody that the first limited portion is  
10 covered with.

14. The apparatus of claim 12,  
wherein the membrane sheet includes third limited portion  
disposed within the opening of the absorbent pad, the third limited  
15 portion does not overlap said first and second limited portions,  
the third limited portion being coated with an antibody that is  
different than the antibodies that the first and second limited  
portions are covered with.

20 15. A method for testing blood comprising the following  
steps:

collecting a blood sample;  
adding an anticoagulant to the blood sample;  
adding BSA to the blood sample;

25 placing a blood testing apparatus in the blood sample, the  
blood testing apparatus including

a backing sheet including a front surface thereof,  
the front surface of the backing sheet including a first  
section and a section, a membrane sheet being attached  
30 to the first section of the backing sheet, a first  
limited portion of the membrane sheet being coated with  
an antibody selected from the group consisting of  
antibody for A antigen, antibody for B antigen and  
antibody for RH positive antigen, an absorbent pad being  
35 attached to the section of the backing sheet, the  
membrane sheet and absorbent pad being in juxtaposed  
relationship with one another;

waiting for blood to adhere to the first limited portion of  
the membrane.

16. A method for testing blood comprising the following steps:

collecting a blood sample;

adding an anticoagulant to the blood sample;

5 adding BSA to the blood sample;

placing the blood sample into a blood testing apparatus, the blood testing apparatus including

10 a bottom receptacle including an upper opening defined by an upper rim for accommodating a membrane sheet and an absorbent pad, the membrane sheet overlying the absorbent pad, a first limited portion of the membrane sheet being coated with an antibody selected from the group consisting of antibody for A antigen, antibody for B antigen and antibody for RH positive antigen, the  
15 absorbent pad including an opening, the first limited portion of the membrane sheet being disposed within the opening of the absorbent pad;

waiting for blood to adhere to the first limited portion of the membrane.

20 17. A method for testing blood comprising the following steps:

collecting a blood sample;

adding an anticoagulant to the blood sample;

25 adding BSA to the blood sample;

adding an antibody to the blood sample;

placing a portion of the blood sample onto a membrane;

waiting for blood and antibody to adhere to the membrane or pass through the membrane.

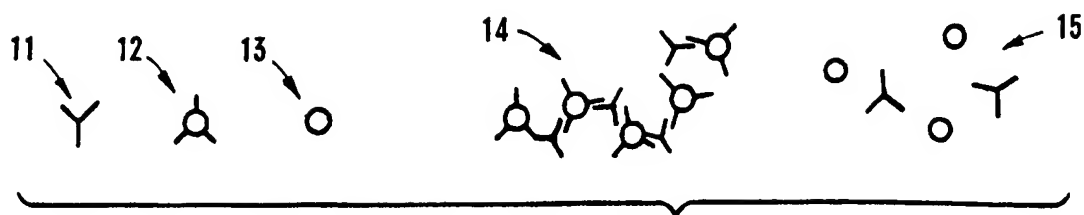


Fig. 1

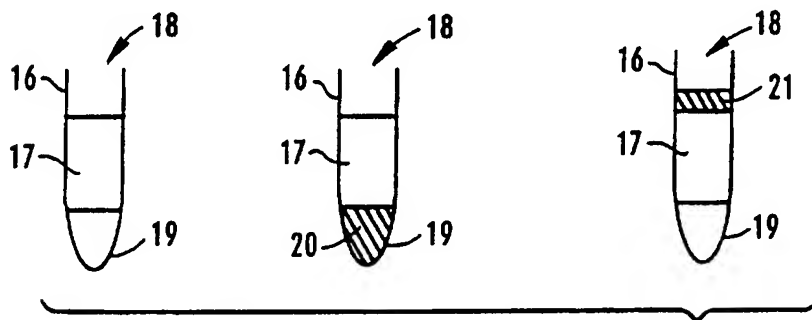


Fig. 2  
(PRIOR ART)



Fig. 3

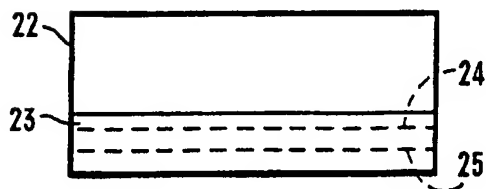


Fig. 4

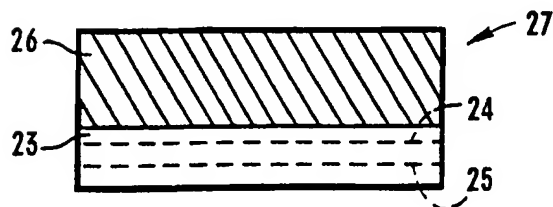


Fig. 5



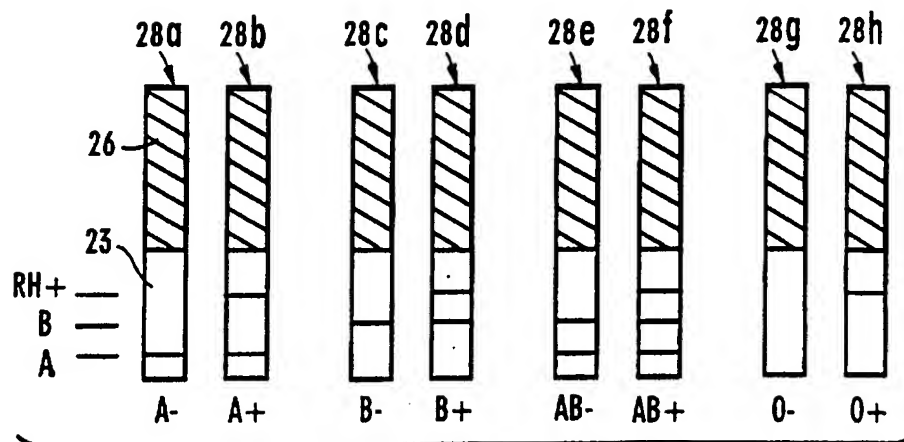


Fig. 6

Fig. 10

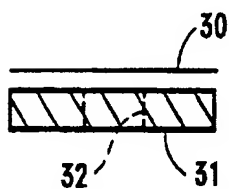


Fig. 7

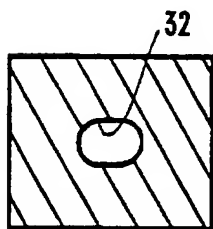


Fig. 8

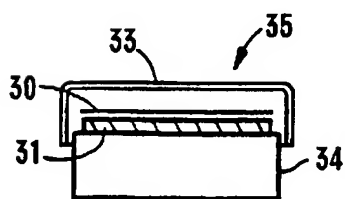
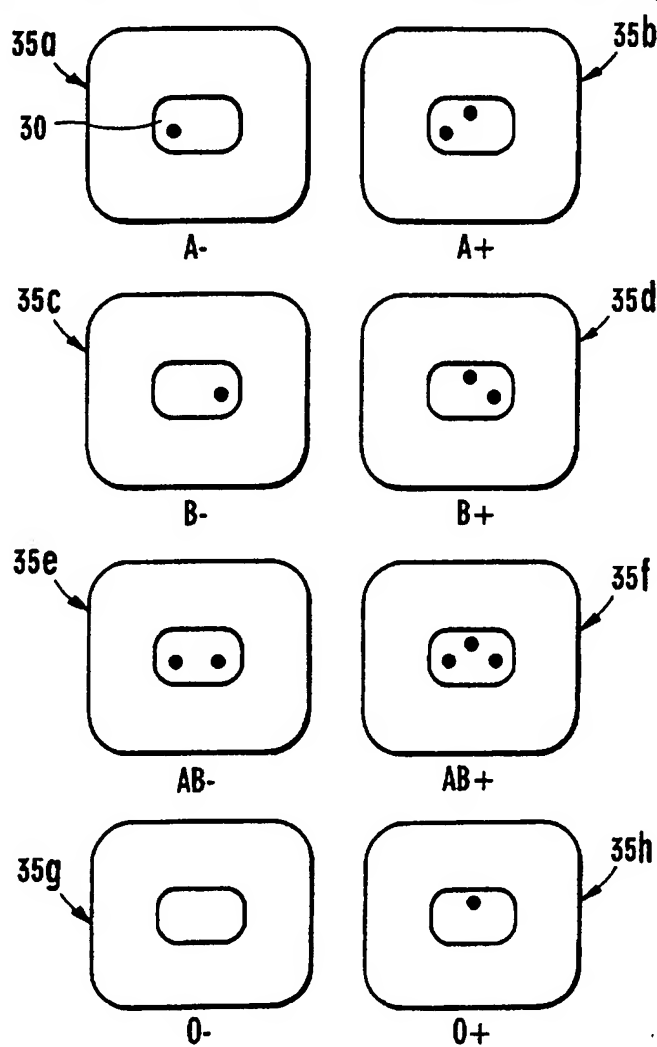


Fig. 9



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